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MODEL STUDIES ON THE FORMATION OF ORGANIC COMPOUNDS IN SIMPLE ATMOSPHERIC GASES BY ELECTRICAL DISCHARGES

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ABSTRACT. Reference is made to the formation of amino acids from simple gas mixtures under the influence of electrical discharges as a model reaction for questions on the composition of the atmosphere in early stages of the Earth's history, in connection with the 'original synthesis' of basic biochemical components. In an atmosphere containing hydrogen sulfide, ammonium thiocyanate forms, along with two still unidentified sulfur-containing compounds. Amino acid synthesis is not markedly affected by the presence of hydrogen sulfide. Under certain conditions, at higher reaction temperatures, compounds with guanido groups form, such as glycocyamine. The volatile reaction products resulting from the thermal decomposition of amino acids serve for resynthesis of the simplest representatives of the amino acids if oxygen is excluded, and with appropriate addition of energy.

Introduction

In studies on the thermal conversion products of amino acids [1], water, /385 ammonia, hydrocyanic acid and carbon dioxide were detected as volatile decomposition products. This suggested the question of the conditions under which synthesis of amino acids from these simple "decomposition products" is possible. This problem is also of considerable interest in connection with ideas about the basic chemical processes in early stages of the Earth's history.

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*Numbers in the margin indicate pagination in the original foreign text.

Berthelot [2] has already undertaken experiments attempting to form large organic nitrogen-containing molecules from simple alcohols and ethers in the presence of nitrogen by quiet discharges in the gas phase. W. Löb was the first to realize that a reducing atmosphere is necessary for the formation of glycine by the action of dark electrical discharges on simple gas mixtures (especially water, ammonia, and carbon monoxide). Independently of these authors, who received no attention for a long time, suggestions have also been made by other authors, including Oparin [4], Urey [5], Quiring [6], and Kuhn [7], on the early development of the Earth's atmosphere and its relation with the "original synthesis" of organic chemical materials as basic bio-chemical components.

A considerable part of these suggestions are of a hypothetical character, and are not susceptible to direct experimental confirmation. The direct experimental methods by means of which one can obtain information on these questions have recently been enlarged by a promising method.

Miller [8] has produced an apparatus to simulate the ideas advocated by Urey [5] on the development of the Earth's atmosphere. With this apparatus, model experiments can be undertaken to test hypotheses on conditions in the early atmosphere of the Earth. In this way progress has been made again, with new objectives, along the path described by Berthelot [2]. No progress had been made in that direction after the unjustly forgotten experiments of Löb [3] and some very quickly terminated technical achievements by Gluud [9] on the production of glycine from blast furnace gases.

Our Own Model Experiments

The model experiments are based on the following deliberations: Formation of low-molecular-weight organic materials, which characterize the early stage of chemical evolution in the sense of Calvin [10], very probably occurred in the atmosphere, or in any case with its decisive participation. Therefore, only such an atmospheric composition as permits the formation of simple
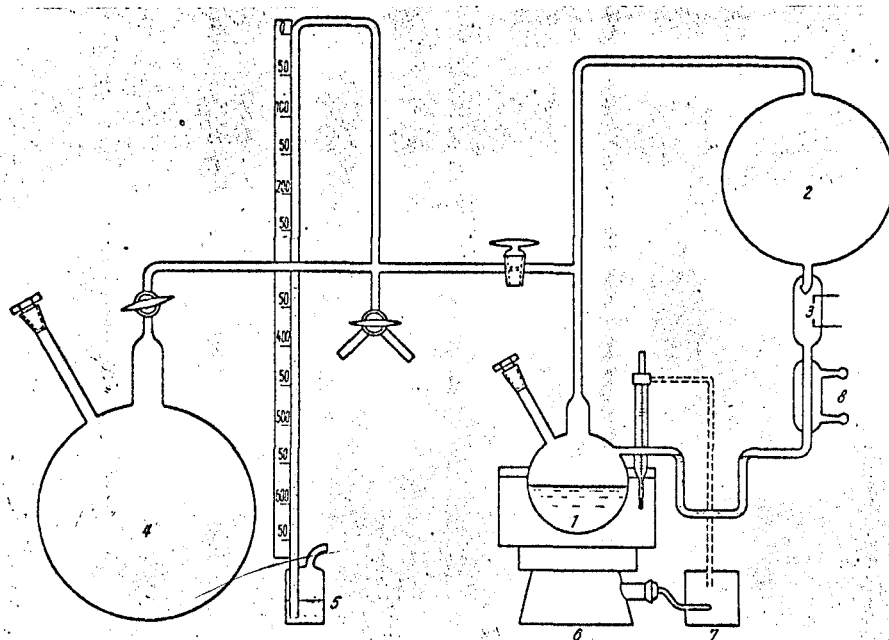
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Figure 1. Circulation system used for the model experiments.

1 - flask for the aqueous phase (model ocean); 2 - gas
container (model atmosphere); 3 - sparking section; 4 -
supply container; 5 - mercury manometer; 6 - hot plate;
7 - relay; 8 - cooler.

organic molecules such as amino acids with appropriate addition of energy in
a model experiment can be considered as probable for the early periods of our
planet. Like Miller, we have selected electrical discharge as the form of the
energy source, and we have proceeded from the concept that the organic com-
pounds formed in the layers of the atmosphere near the Earth reached the
ocean in the rain. Figure 1 shows the apparatus which we used. The model
ocean is contained in flask 1. The ocean is represented by 100 cm^3 of water.
The model atmosphere, of variable composition, is contained in gas-holder 2.
The energy source is in the spark section 3. Material transport is effected
by the boiling aqueous phase and by condensation of the vapor in the cooler
connected after the spark section.

As a criterion for the formation of low-molecular-weight organic com-
pounds, we used the determination of amino acids. The formation of amino acids

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TABLE 1. GAS MIXTURES TESTED AS MODEL ATMOSPHERES

Experi- ment	Composition of the atmosphere[1] (percent by volume)						Ninhydrin- positive substances
	CH_4	CO_2	NH_3	N_2	H_2	O_2	
1	40	—	40	—	20	—	+
2	40	—	40	16	—	4	+
3	6.7	53.3	26.7	13.3	—	—	+
4	—	50	—	50	—	—	—
5	—	50	50	—	—	—	+

[1]The composition introduced into the stock container in the apparatus is given. The amount of water vapor in the apparatus must be added.

must be considered as one of the most important processes of early chemical evolution. Some of the model atmospheres which we tested are summarized in Table 1.

The composition of the atmosphere in Experiment 1 corresponds to that employed by Miller [8]. In the course of our experiments we were able, among other things, to confirm the result of that author with respect to the amino acids produced. The following amino acids were identified by two-dimensional paper chromatography: glycine, α-alanine, β-alanine, sarcosine, α-aminobutyric acid.

Experiment 2 was intended to answer the question of whether amino acids are formed in the presence of oxygen. In this case the oxygen concentration was limited by the explosive limits for oxygen-methane mixtures. Mass spectrometric gas analysis of the model atmosphere after the end of the experiment showed that the oxygen was practically used up. From the argon: oxygen ratio, a drop in oxygen concentration from 4% to 0.0067% could be calculated. The CO_2 content of the model atmosphere increased from 0.006 to

1.7%. Therefore, the major amount of the oxygen was converted according to the equation

$$\tfrac{1}{2} CH_4 + O_2 = \tfrac{1}{2} CO_2 + H_2O \qquad (1)$$

This observation is noteworthy insofar as one would expect the reaction

$$\tfrac{4}{3} NH_3 + O_2 = \tfrac{2}{3} N_2 + 2 H_2O \qquad (2)$$

on the basis of considerations by Urey [5], because there is a considerably greater decrease in free energy with reaction 2 than with reaction 1.

$$(2: \Delta F^\circ_{298} = -110{,}73 \text{ kcal} \quad 1: \Delta F^\circ_{298} = -97{,}75 \text{ kcal}).$$

Experiments 3 and 4 were intended to make an experimental contribution toward a hypothesis proposed by Quiring [6] on the composition of the primordial atmosphere of the Earth. Quiring [6] advocates the view that the primordial atmosphere consisted primarily of nitrogen and carbon dioxide (along with the water vapor always present for the original syntheses). According to his concept, ammonia and methane appeared only in traces. Experiment 3 shows that amino acids are formed from an atmosphere which contains ammonia and methane in significant amounts along with carbon dioxide and nitrogen.

Experiment 4, in contrast, shows that no amino acids are formed in the model system we have selected, if only CO_2 and nitrogen are in the atmosphere along with water vapor.

Experiment 5 shows that amino acids are formed if the nitrogen in the atmosphere of the previous model experiment is replaced by ammonia. This result also confirms the formation of glycine from carbon dioxide and ammonia, which was conjectured by Löb [2], but which could not be established unambiguously with the methods then available.

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TABLE 2. R_F VALUES OF THE SUBSTANCES PRODUCED IN THE MODEL
EXPERIMENTS WITH HYDROGEN SULFIDE-CONTAINING ATMOSPHERES
WHICH BLEACH THE IODINE AZIDE REAGENT

Substance	R_F value
1	0.04
2	0.22
3	0.35
Thiourea	0.45
Thioacetamide	0.75

Paper: Schleicher & Schüll 602 h:p; solvent: butanol:acetic acid:water (70:7:23).

As Urey considers considerable enrichment with ammonium sulfide possible in very early periods of the Earth's history ([5], p. 133), we have tested the effect of the presence of hydrogen sulfide on the model reaction of hydrogen, ammonia, and methane (in the composition of Experiment 1, Table 1). For these experiments, vessel 1 of the circulation system was charged with water saturated with hydrogen sulfide.

In the beginning, three substances which bleached the iodine azide reagent of Raschig [11] were detected. The R_F values for these compounds, as well as those of some comparison materials, are listed in Table 2. Compound 3, which gave a red-brown spot on the paper chromatogram after spraying with ferric chloride solution, could be isolated after removal of the amino acids with an ion exchanger (Dowex 50). The substance could be precipitated from aqueous solution with silver nitrate, and could be purified by solution of the precipitate and reprecipitation. The silver salt was identified as silver thiocyanate by means of its infrared spectrum. Ammonium thiocyanate and substance 3 give identical R_F values on the paper chromatogram. Formation of thiocyanate is also observed if hydrogen sulfide is left out of the model

TABLE 3. COMPARISON OF THE FORMATION OF VARIOUS AMINO ACIDS
FROM AN ATMOSPHERE OF METHANE, AMMONIA AND HYDROGEN
(EXPERIMENT 1, TABLE 1) WITH OR WITHOUT HYDROGEN SULFIDE,
REFERRED TO GLYCINE AS 1.

Amino acids	Without H_2S addition			With H_2S addition
	1	2	3	
1. Glycine	1.00	1.00.	1.00	1.00
2. Alanine	0.54	0.65	0.11	0.23
3. Sarcosine	0.08	0.04	1.07	1.26
4. β-alanine	0.24	0.33	0.05	0.15
5. α-aminobutyric acid	0.08	0.054	0.01	
6. B_1*)	0.02	0.002	0.16	0.34
7. B_2*)	0.05	0.007	0.18	

*) Amino acids B_1 and B_2 could not be identified by Miller because their quantity was too small; thus, the values listed in the table are estimates.

The values which we found for the sum: α-aminobutyric acid + B_1 + B_2 were calculated as about 325 to 350 ml eluate from the two bands which were not separated (cf. Figure 2). Lines 5, 6, and 7 must not be considered because of the uncertainties described.

atmosphere, and instead, sulfer is added to the model ocean. No ammonium thiocyanate forms without the electrical discharge. We are still working on the identification of compounds 1 and 2.

Quantitative determination of the amino acids formed in the presence of hydrogen sulfide, by the method of Stein and Moore [12] (cf. Figure 2) allowed comparison of our experimental results with those of the pure methane-ammonia-hydrogen atmosphere according to Miller (columns 1 to 3 of Table 3) [8].
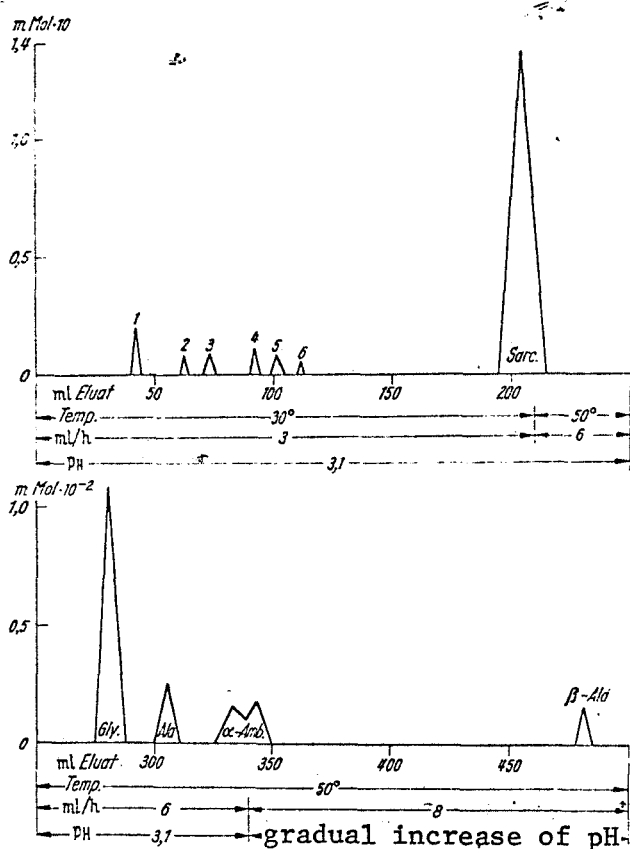
Figure 2. Chromatogram, by the procedure of Stein and Moore [12], of the amino acids produced from ammonia, methane, and hydrogen in the presence of hydrogen sulfide. Peaks 1 to 6 were not studied because the amounts of material were too small. Sarc.: sarcosine; gly.: glycine; ala.: alanine; α-amb.: α-aminobutyric acid.

Under certain conditions, the details of which will be considered later, substances containing the guanido group are obtained along with amino acids if the temperature of the heating bath is raised to 200° for the same duration of experiment. Of these compounds, one substance was identified as glycocyamine by paper chromatography.

Discussion of the Results

With the experimental findings described above, the observation of such a clear and frankly expressed tendency for production of amino acids from gaseous atmospheres appears to us to be so remarkable that no one can suggest that such reactions did not occur as soon as, and as long as, the hypothesized composition of the Earth's atmosphere was present. But in such a case we must expect a considerable yield of organic substances. In agreement with Miller, we obtained some 100 mg of amino acids from a gas volume of 3 liters (gas container 2 of Figure 1) at a pressure of 260 mm in the course of 7 days. The fact must be considered that such an effective synthesis participated in the further changes to which the primitive atmosphere of our planet was subjected. Considering the notable reproducibility of this reaction even with respect to the quantitative relations, in which we see the effect of a general synthetic principle, we believe that we can justify some careful conclusions. On the basis of the
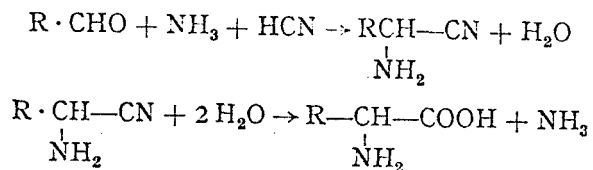
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results reported, the conclusion is reached that the atmosphere of the Earth at the period when the low-molecular-weight organic molecules were being formed, and the protein monomers in particular, apparently showed reducing properties. The result of Experiment 4, Table 1, shows that in a nonreducing atmosphere the formation of amino acids, otherwise unavoidable, did not take place. On the basis of our model, then, we consider it improbable that the Earth had such a nonreducing atmosphere during the period of amino acid formation, if the atmosphere is conceded an important role in the formation of these substances.

For the reaction of methane and ammonia with oxygen in the electric discharge (Table 1, Experiment 2), the compound for which the greatest amount of energy is released in its formation does not occur. This observation shows that the opinion advocated by Urey ([5], p. 151), that reactions proceeded under equilibrium conditions in the primitive atmosphere, is not supported by the model experiment. Obviously, thermodynamic equilibrium is not attained in the model experiment, so that no predictions can be made from the change of free energy as to which reaction is actually proceeding. In this region also, the evidence which can be obtained from model reactions appears suitable for deeper penetration into the conditions of the early atmosphere of the Earth.

Comparison of amounts of amino acids obtained with addition of hydrogen sulfide and the amounts obtained in its absence shows that these values are equal within the range of error due to the apparatus, as expressed in Experiments 1, 2, and 3. Therefore, the presence of hydrogen sulfide in the primitive Earth atmosphere did not markedly affect the formation of amino acids. Formation of sulfur-containing amino acids has not yet been demonstrated.

The mechanism for the formation of the amino acids is not yet clear in <u>/388</u> its details. Miller [8] discusses a series of hypotheses for the mechanism of formation. According to one of these concepts, aldehydes and hydrogen cyanide,

formed in the sparking section, represent intermediate stages of the synthesis, so that formation of amino acids by a cyanohydrin synthesis would be conceivable:

$$R \cdot CHO + NH_3 + HCN \rightarrow \underset{\underset{NH_2}{|}}{RCH} - CN + H_2O$$

$$\underset{\underset{NH_2}{|}}{R \cdot CH} - CN + 2\,H_2O \rightarrow \underset{\underset{NH_2}{|}}{R} - CH - COOH + NH_3$$

Formation of thiocyanate in the presence of H_2S or sulfur can be explained on the basis of this concept, as thiocyanate is easily obtained from cyanides by combination with sulfur.

The possibility of occurrence of substances containing the guanido group thus appears particularly noteworthy to us, because this directly gives a connection with the components of nucleic acids.

Let us return to the starting point of this discussion, the consideration of hydrogen cyanide. The formation of thiocyanates supports its intermediate occurrence in the formation of amino acids from simple gases. We find that the gaseous thermal decomposition products of amino acids, with oxygen excluded, which were observed previously, can lead to resynthesis of the simplest amino acids with appropriate supply of energy.

Experimental Part

Flask 1 of the apparatus shown in Figure 1 was filled with 100 ml water, and the apparatus was evacuated until the water boiled. In order to remove oxygen, hydrogen was let in and the apparatus was again evacuated. After two repetitions of this process, the supply vessel was filled, for example,

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with one of the mixtures given in Table 1. The gas was taken from steel bottles without previous purification. The heating bath was now brought to a temperature of 120° and the circulation system was filled with the gas mixture in the supply vessel to a total pressure of 260 Torr, and the sparking section was turned on. The experiment lasted one week.

Gas analysis of the gas mixture obtained from Experiment 2 of Table 1 was done in a mass spectrometer, Type IS, of the Atlas Works in Bremen [13]. The value of 0.006% CO_2 for the gas mixture 1 of Table 1 was calculated from the proportion of air contained in this mixture. The paper-chromatographic studies were performed in the manner described previously [14].

Quantitative Analysis of the Amino Acids

The amino acids which formed in the presence of hydrogen sulfide were determined by use of the Stein and Moore method [12]. An extensive description of the methodology we used appears briefly elsewhere [15].

Dowex 50X4 was used as the ion exchanger. For separation of the fractions, we used the Serva Fraction Collector of Zentralwerkstatt Göttingen, Inc., connected as a drop counter. We used wetting agent VP 3241 to obtain a constant drop size[1]. As a check on the drop counting, every tenth test tube was weighed.

Reagents. Ninhydrin: For spectrophotometric measurements it is advisable to recrystallize the commercial ninhydrin from 1 N hydrochloric acid, according to Hamilton [16], in order to get low blank readings. The ordinary commercial ninhydrin often gives blank absorbances of 0.2 to 0.3 under the conditions of the analysis. By recrystallization, one obtains preparations giving blanks of 0.09 to 0.14.

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[1]We thank Anorgana, Inc., Munich, for generously providing the wetting agent.

Hydrindantin was obtained in sufficient purity by reduction of ninhydrin with ascorbic acid [12]. Methyl cellosolve (ethylene glycol monomethyl ether) was distilled and used if the distillate gave no color with freshly prepared 4% KI solution. The reagent solution for photometric determination of the amino acids was prepared in the following manner directly before use: 1 g ninhydrin and 0.15 g hydrindantin were dissolved in 37.5 ml methyl cellosolve with 12.5 ml 4 M citrate buffer, pH 5.5 (according to Stein and Moore [12]).

In the preparatory work for amino acid determination, an unexpected source of error must first be eliminated. Knowledge of it saves time-consuming preliminary experiments in use of the amino acid testing procedure of Stein and Moore, and thus we would like to report it.

At first we could not obtain any reproducible amino acid values in setting up the calibration curve for leucine. On systematic studies to uncover the source of error, it appeared that it was in the composition of the test tubes used for the studies (Fiolax, Schott & Gen.). With otherwise identical conditions, quite reproducible results could be obtained in quartz tubes. For the further studies, we used Elka test tubes from the Karl Hecht glassware factory, Sontheim an der Rhön. They did not show the effect observed with the Fiolax tubes.

Performance of the analyses. The reaction solution produced in the model experiment was treated with barium hydroxide solution until the reaction was strongly alkaline. This solution was flushed with ammonia-free air until no more ammonia could be detected in a wash bottle of Nessler's reagent connected after the solution. The ammonia-free solution was brought to pH2 with 2N sulfuric acid, and the barium sulfate was filtered off. Kjekdahl nitrogen determination in our parallel experiment showed a nitrogen content of 14.8 mg for the total product.

In the first analysis, only every other test tube was boiled with the /389 reagent solution. The content of the other tubes was closely defined for the purpose of paper-chromatographic analysis. In this way, sarcosine, glycine,

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alanine, α-aminobutyric acid and β-alanine were detected. The peak from α-aminobutyric acid was very wide, and showed two points. It contained still other amino acids, which we have not yet been able to identify. For determination of the amino acids present in very small concentration, each test tube was boiled with the reagent solution in the second analysis. In the fractionation range between 500 and 850 ml of eluate, no more amino acids were detected. The amino acids in the six maxima appearing between 40 and 115 ml could not be identified because the quantities were too small. The first of these maxima corresponds to the volume of the column which is taken up by the buffer (40 to 44 ml). The compounds appearing in this maximum are presumed to be polymeric compounds which are not retained by the column.

TABLE 4. QUANTITATIVE DETERMINATION OF THE AMINO ACIDS
IDENTIFIABLE BY PAPER CHROMATOGRAPHY, ARISING FROM
A SULFUR-CONTAINING MODEL ATMOSPHERE

Amino acids	Sarc.	Gly.	Ala.	α-Amb.	α-Ala.
mMol $\cdot$ 10^{-2}	1.37	1.09	0.25	0.37	0.159
Relative to glycine = 1 ..	1.26	1.00	0.23	0.34	0.15

The quantitative results for the identifiable amino acids are shown in Table 4.

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